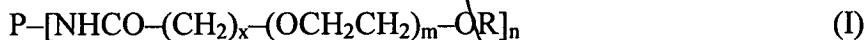


What is claimed is:

1. A conjugate, said conjugate comprising an erythropoietin glycoprotein having at least one free amino group and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to "n" poly(ethylene glycol) groups of the formula $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$ with the -CO of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; wherein R is lower alkyl; x is 2 or 3; m is from about 450 to about 900; n is from 1 to 3; and n and m are chosen so that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons.

15 2. The conjugate of claim 1, of the formula:



wherein m, n and R are as above, and P is the residue of the glycoprotein without the n amino group(s) which form amide linkage(s) with the poly(ethylene glycol) group(s).

3. The conjugate of claim 2, wherein the glycoprotein is a human erythropoietin.

4. The conjugate of claim 3, wherein the human erythropoietin glycoprotein is expressed by endogenous gene activation.

5. The conjugate of claim 3, wherein the glycoprotein has the sequence SEQ ID NO:1.

30 6. The conjugate of claim 5, wherein R is methyl.

7. The conjugate of claim 5, wherein m is from about 650 to about 750.

8. The conjugate of claim 5, wherein n is 1.

5 9. The conjugate of claim 5, wherein R is methyl; m is from about 650 to about 750; and n is 1.

10 10. The conjugate of claim 2, wherein the glycoprotein has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.

11. The conjugate of claim 10, wherein the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:

Asn³⁰Thr³²;

Asn⁵¹Thr⁵³,

15 Asn⁵⁷Thr⁵⁹;

Asn⁶⁹;

Asn⁶⁹Thr⁷¹;

Ser⁶⁸Asn⁶⁹Thr⁷¹;

Val⁸⁷Asn⁸⁸Thr⁹⁰;

20 Ser⁸⁷Asn⁸⁸Thr⁹⁰;

Ser⁸⁷Asn⁸⁸Gly⁸⁹Thr⁹⁰;

Ser⁸⁷Asn⁸⁸Thr⁹⁰Thr⁹²;

Ser⁸⁷Asn⁸⁸Thr⁹⁰Ala¹⁶²;

Asn⁶⁹Thr⁷¹Ser⁸⁷Asn⁸⁸Thr⁹⁰;

25 Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰;

Asn⁸⁹Ile⁹⁰Thr⁹¹;

Ser⁸⁷Asn⁸⁹Ile⁹⁰Thr⁹¹;

Asn¹³⁶Thr¹³⁸;

Asn¹³⁸Thr¹⁴⁰;

30 Thr¹²⁵; and

Pro¹²⁴Thr¹²⁵.

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22. The conjugate of claim 18, wherein R is methyl; m is from about 650 to about 750; and n is 1.

23. The conjugate of claim 2, wherein the glycoprotein has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

13 24. The conjugate of claim 23, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin and addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

10 14 13 25. The conjugate of claim 24, wherein the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:
Gln²⁴ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰;
Gln³⁸ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰; and
15 Gln⁸³ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰.

15 26. The conjugate of claim 25, wherein R is methyl.

27. The conjugate of claim 25, wherein m is from about 650 to about 750.

20 28. The conjugate of claim 25, wherein n is 1.

29. The conjugate of claim 25, wherein R is methyl; m is from about 650 to about 750; and n is 1.

25 30. A composition comprising conjugates, each of said conjugates comprising an erythropoietin glycoprotein having at least one free amino group and having the *in vivo* biological activity of causing bone marrow cells to increase production of reticulocytes and red blood cells and selected from the group consisting of human erythropoietin and analogs thereof which have sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; the

12. The conjugate of claim 11, wherein R is methyl.

13. The conjugate of claim 11, wherein m is from about 650 to about 750.

5 14. The conjugate of claim 11, wherein n is 1.

15. The conjugate of claim 11, wherein R is methyl; m is from about 650 to about 750; and n is 1.

10 16. The conjugate of claim 10, wherein the glycoprotein has a sequence comprising the sequence of human erythropoietin and a second sequence at the carboxy terminus of the human erythropoietin sequence, wherein the second sequence contains at least one glycosylation site.

15 17. The conjugate of claim 16, wherein the second sequences comprises a sequence derived from the carboxy terminal sequence of human chorionic gonadotropin.

18. The conjugate of claim 17, wherein the glycoprotein has a sequence selected from the group consisting of:

20 (a) the sequence of human erythropoietin and the sequence SEQ ID NO:3 at the carboxy terminus of the human erythropoietin sequence;

(b) the sequence in (a) modified by Ser⁸⁷ Asn⁸⁸ Thr⁹⁰; and

(c) the sequence in (a) modified by Asn³⁰ Thr³² Val⁸⁷ Asn⁸⁸ Thr⁹⁰.

25 19. The conjugate of claim 18, wherein R is methyl.

20. The conjugate of claim 18, wherein m is from about 650 to about 750.

21. The conjugate of claim 18, wherein n is 1.

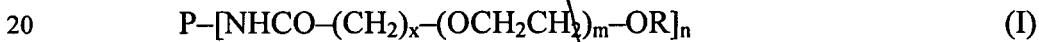
glycoprotein in each said conjugate being covalently linked to "n" poly(ethylene glycol) groups of the formula $-\text{CO}-(\text{CH}_2)_x-(\text{OCH}_2\text{CH}_2)_m-\text{OR}$ with the -CO of each poly(ethylene glycol) group forming an amide bond with one of said amino groups; where in each of said conjugates R is lower alkyl; x is 2 or 3; m is from about 450 to 5 about 900; n is from 1 to 3; n and m are chosen so that the molecular weight of each conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons; the percentage of conjugates where n is 1 is at least ninety percent.

31. The composition of claim 30 wherein the percentage of conjugates where n is 1
10 is at least ninety-two percent.

32. The composition of claim 31 wherein the percentage of conjugates where n is 1
is at least ninety-six percent.

15 33. The composition of claim 30 wherein the percentage of conjugates where n is 1
is from ninety percent to ninety-six percent.

34. The composition of claim 30, wherein each of said conjugates has the formula:



wherein m, n and R are as above, and P in each conjugate is the residue of the glycoprotein without the n amino group(s) which form amide linkage(s) with the poly(ethylene glycol) group(s).

25 35. The composition of claim 34 wherein the percentage of conjugates where n is 1
is at least ninety-two percent.

30 36. The composition of claim 35 wherein the percentage of conjugates where n is 1
is at least ninety-six percent.

37. The composition of claim 34 wherein the percentage of conjugates where n is 1 is from ninety percent to ninety-six percent.

38. The composition of claim 34, where in each conjugate the glycoprotein is a 5 human erythropoietin.

39. The composition of claim 38, where in each conjugate the human erythropoietin glycoprotein is expressed by endogenous gene activation.

10 40. The composition of claim 38, where in each conjugate the glycoprotein has the sequence SEQ ID NO:1.

41. The composition of claim 40, where in each conjugate R is methyl.

15 42. The composition of claim 40, where in each conjugate m is from about 650 to about 750.

43. The composition of claim 40, where in each conjugate R is methyl and m is from about 650 to about 750.

20 44. The composition of claim 34, where in each conjugate the glycoprotein has the sequence of human erythropoietin modified by the addition of from 1 to 6 glycosylation sites.

25 45. The composition of claim 44, where in each conjugate the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:
Asn³⁰Thr³²;
Asn⁵¹Thr⁵³,
30 Asn⁵⁷Thr⁵⁹;
Asn⁶⁹;

Asn⁶⁹Thr⁷¹;
Ser⁶⁸Asn⁶⁹Thr⁷¹;
Val⁸⁷Asn⁸⁸Thr⁹⁰;
Ser⁸⁷Asn⁸⁸Thr⁹⁰;
5 Ser⁸⁷Asn⁸⁸Gly⁸⁹Thr⁹⁰;
Ser⁸⁷Asn⁸⁸Thr⁹⁰Thr⁹²;
Ser⁸⁷Asn⁸⁸Thr⁹⁰Ala¹⁶²;
Asn⁶⁹Thr⁷¹Ser⁸⁷Asn⁸⁸Thr⁹⁰;
Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰;
10 Asn⁸⁹Ile⁹⁰Thr⁹¹;
Ser⁸⁷Asn⁸⁹Ile⁹⁰Thr⁹¹;
Asn¹³⁶Thr¹³⁸;
Asn¹³⁸Thr¹⁴⁰;
Thr¹²⁵; and
15 Pro¹²⁴Thr¹²⁵.

46. The composition of claim 45, where in each conjugate R is methyl.

47. The composition of claim 45, where in each conjugate m is from about 650 to
20 about 750.

48. The composition of claim 45, where in each conjugate R is methyl and m is from
about 650 to about 750.

25 49. The composition of claim 44, where in each conjugate the glycoprotein has a
sequence comprising the sequence of human erythropoietin and a second sequence at the
carboxy terminus of the human erythropoietin sequence, wherein the second sequence
contains at least one glycosylation site.

50. The composition of claim 49, where in each conjugate the second sequences comprises a sequence derived from the carboxy terminal sequence of human chorionic gonadotropin.

5 51. The composition of claim 50, where in each conjugate the glycoprotein has a sequence selected from the group consisting of:

(a) the sequence of human erythropoietin and the sequence SEQ ID NO:3 at the carboxy terminus of the human erythropoietin sequence;

(b) the sequence in (a) modified by Ser⁸⁷ Asn⁸⁸ Thr⁹⁰; and

10 (c) the sequence in (a) modified by Asn³⁰ Thr³² Val⁸⁷ Asn⁸⁸ Thr⁹⁰.

52. The composition of claim 51, where in each conjugate R is methyl.

53. The composition of claim 51, where in each conjugate m is from about 650 to
15 about 750.

54. The composition of claim 51, where in each conjugate R is methyl and m is from about 650 to about 750.

20 55. The composition of claim 34, where in each conjugate the glycoprotein has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.

25 56. The composition of claim 55, where in each conjugate the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin and addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.

30 57. The composition of claim 56, where in each conjugate the glycoprotein has the sequence of human erythropoietin modified by a modification selected from the group consisting of:

~~Gln²⁴ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰;~~
~~Gln³⁸ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰; and~~
~~Gln⁸³ Ser⁸⁷ Asn⁸⁸ Thr⁹⁰.~~

5 58. The composition of claim 57, where in each conjugate R is methyl.

59. The composition of claim 57, where in each conjugate m is from about 650 to about 750.

10 60. The composition of claim 57, where in each conjugate R is methyl and m is from about 650 to about 750.

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